Back of book T.R.P. Ross

FRANCE (+ England)
1973. Bruyzoan + field notes.

Simpson

Stenographer's Note Book

GREGG RULED

No. 2071 GG

Simpson Lee Paper Company
Everett, Wash.

1PAR.003198

32. Heterozooids (& Cenozooids)
34. Avicularium on stained Bugula
35. Zooid anatomy
36. Pore plates
37. Muscle topography in zooid.
38. Vestibular glands Seasonality in bryozoans.
39. Budding in Membraniporea
40. Attachment of funiculus; Position of anus.
41. Vestibular glands; tentacle glands
42. Spermatogenesis & oogenesis. Intervals of fertilization
43. Fixation & staining
44. Egg development (cleavage); larvae in ovicells, pseudopores
45. Slides examined in Paris - vestibular glands; brown body
46-52 anus; pore plates; bacterial glands; Progression in vestibular glands. Development of spines
47. Bacterial gland; regeneration; spermatozoa
48. Funiculus; egg development
49. Fixation; decalcification
50 Vestibular & bacterial glands
51 Pore plates; glands; muscles
52. pore plates. E. Niphadographa.

$C_{10}H_{20}O$

Pages. Make an Index for this French Study.

1-2,4 Bryozoan walls
3 Keeping b. alive in lab
3. Ref. to gen. anatomy
4. Spp. found in dredged sample from Ile Louet.
5. Differentiation - dedifferentiation in b.
6. Effects of oil pollution on bry.
6. Spine fm. in bry.
7-8 Embryological dev. in bry.
9. Fixation
9 Ectoderm in bry.
10 Dimorphism. Hybridization. Field Trip to St Pol
11.-14,15 Differentiation of epithelial + peritoneum layers
14,15,17 Funicular strand
16,17 Polypide formation
18-19 Staining + viewing specimens. Methylene blue (also discussion about funiculus) cresyl blue stain. Bryoz. anatomy
20. Spherular leucocytes, pigments in body wall; funiculus brown bodies
21-22 Vibracula; epithelial + peritoneum tissue; pore plates, avicularium
23 Diaphragm. Menthol xls to relax bry. Mounting slides
Tentacular sheath
24. Incomplete desc. of process involved w. methylene blue
25. Caecum; spines.
26+29. Stains. Rouge neutre. Cresyl blue. Methylene blue.
27. Features of cyclostome tentacles.
28. Film. Growth of Membranipora membranacea.
30. Digestive tract.
31. E.M. sucrose-cacodylate "holding" solution.

at least 3

cl. cuticle

What is tentacle sheath attached to??

From where domiciliu

Network funicular strands going through lateral & transverse to form a reticulum throughout the colony.

Does tentacle sheath line coelom

Where colonies abut they only grow together

DATE

$C_{10}H_{16}O$

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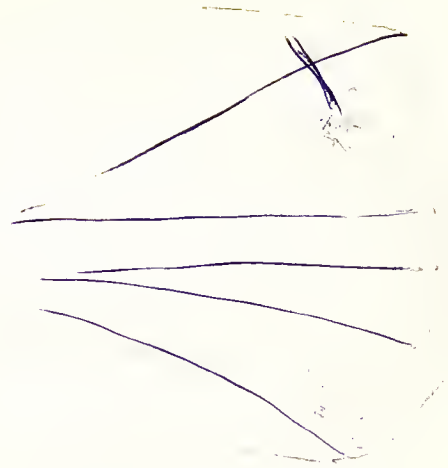
What is tentacle sheath attached to??

at least 30 + funicular strands



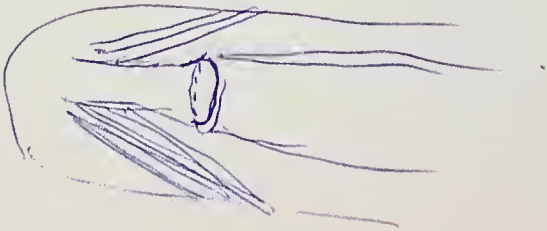
From where do muscles arise.

Network funicular strands going through lateral + transverse walls to form a stratum throughout the colony.

blue funicular strands linking onto tentacle sheath + epaxonium. Also from epaxonium across to wall. Pencil outlines tentacle sheath + diaphragm.

Does tentacle sheath line epaxonium?

When colonies start they only grow together. Differentiation of cells has not yet begun



Discussion w. Geneviève

Tentacles are all the same in cotoproects. V. definite re all cheilostomes. Why not look at cyclostome tentacles. Pedicellarian tentacles might be a useful aspect to examine H. Smith + G. Lutaud - tentacles.

Ectoprocts + entoprocts are v. separate + do not belong together - eg. just look at the tentacles. The tentacles are not at all the same.

D. Gordon's work on digestive tract.

G. uses EDTA to decalcify!

G. says that knifemaker makes too soft a knife for bryozoans + one needs to use punchers on glass as you can't thoroughly decalcify bry. + also there are sand grains wh. do not dissolve.

En route to Roscoff. G. says need hard resin for bry. eg. araldite (didn't hear her correctly). She is going to try Spurr because complete penetration of tissue is a problem.

Off hab. Sept.

G. uses different mounting media depending on stain; gen. C. balsam; dislikes synthetics such as Cedrex; however, admits it is useful under certain conditions.

Schizoporella unicornis - { Johnston types in B.M.
{ Hincks illustration.

(Note from K. McC. S. u. f. vs. S. u. s.s.

S. u. f. - more coarsely perforate - pores larger?
apert - smaller, sinus smaller, more V'd
ovicell smoother, appears less "detached"
avic. possibly little larger - mandible
more setose but rarer - usually
unpaired.
Multilaminar condition rarer in S. u. s.s.
if occurs - then at points of "stress" i.e.
buckling of colony [see Hoodsport material]
No multiple ovicells seen in s. u. f.

A.C. Neville
1967. Biol. Review 42: 421.

1971. J. Cell Sci 8: 95-109.
A.C.N & S.M. Luke.
A biol. system producing a self-assembling cholestric protein liquid crystal.

1969. N&L. A 2 system model for chitin-protein complexes in insect cuticle. Tissue & Cell 1: 689-707.

Molecular architecture of adult locust cuticle at e.m. level.

J.W.F. Costerton & E.A.C. MacRobbie
Ultrastructure of Nitella translucens in relation to ion transport:
Jour. Experimental Botany 21 (68); 535-542, Aug., 1970.

[Smooth endoplasmic reticulum]
Ion transport in characean cells — tracer ions from the outside solution can reach the vacuole by means of 2 paths.
Endoplasmic reticulum is fast component at subcellular level for ion transport.

Photograph of Roman Arena
Apt. 50 Monge Ave.
Arenes de Lutece
N. part dire 1869
Spent mise 1883-1885
L'ensemble restaure

2.30F Kleenex.

Michelle Matracon

Red blood cells.
25 million/m³
length of life 120 days
when forming 2 m/sec.
each dise (erythrocyte) 7.5 μ

$$3\overline{)11000}\ = 3700$$

$$9$$

$$2000$$

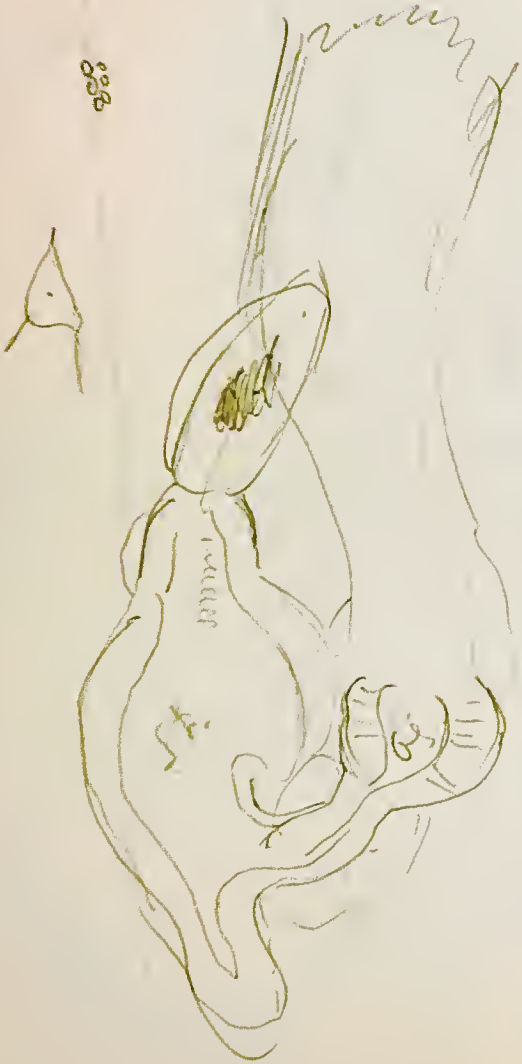
7400

$$3\overline{)5500}\ = 1833$$

$$1833 \times 2 = 3666$$

DATE

S = S



Bulletin Signalétique. Dec 1973. v. 34, no. 6
Abstract listing on Animal Biol. Physio. Path. of
Invert. Zoology

Zoology - Lacaze-Duthiers Library (LD)
Biochemic, Physiol. Yves Delage (YD)

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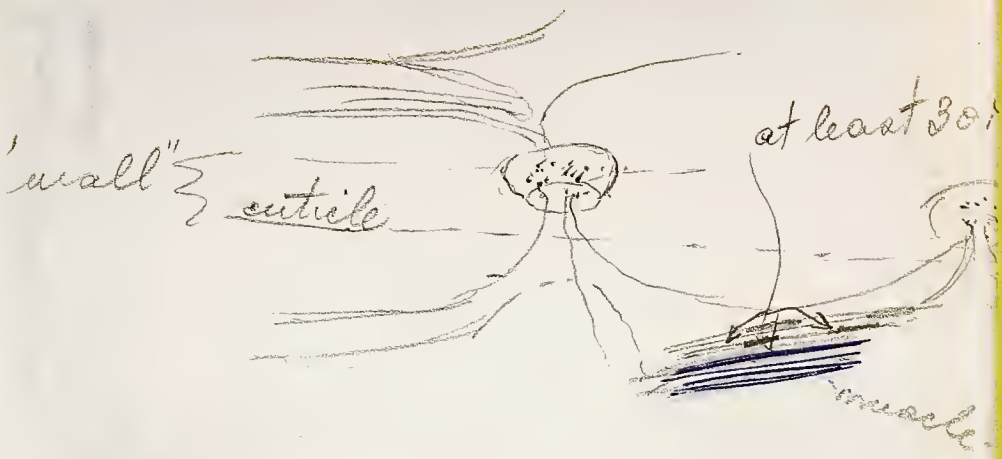

From where do muscles a

Network funicular strands going through lateral & transverse wa to form a reticulum throughout the colony.

Does tentacle sheath line opercu

Where colonies abut they only grow togeth

What is tentacle sheath attached to??

G. Lutaud. 1957. « développement du bourgeon chez M. m (L.)

Bourgeon - bud.
croissance - growth
niveaux - level.

cloison - partition
paroi - wall, side
réseau - network, system
tiers - third, 3rd part
écartement - separation, spacing
de membres - dwelled, stayed

étaler - to spread out

p. 150, line 6, neutral red, (H.2) — usual vital stain used in histology.

p. 150, line 3 in H5 — parietal mesenchyme; "spherular leucocytes."

p. 150, line 2 in H6 — le tiers de la longueur.

p. 150, line 3 in H7

p. 151, line 4 in H1. They never pass from ~~the~~ below the "face" of the colony & remain concentrated.

ANNEE 1972

ADDITIF A LA DOCUMENTATION
P.C.E.M. - PHARMACIE - DENTAIRE

AUTRES PREPARATIONS ET MATIERES DISPONIBLES

PREPARATIONS SUR PLACE :

1ère année de P.C.E.M.	MATHEMATIQUES
Dentaire 2ème année	ANATOMIE PHYSIOLOGIE HISTOLOGIE BIOCHIMIE BIOPHYSIQUE

PREPARATIONS PAR CORRESPONDANCE :

1ère année de P.C.E.M.	MATHEMATIQUES STATISTIQUES
1ère année de Pharmacie	MATHEMATIQUES STATISTIQUES PHYSIQUE CHIMIE GENERALE et ORGANIQUE

Field Locality. Environs Roscoff.

Sept. 13, 1973. Pointe St. Anne. Black buoy N of this Pointe at low tide. Baie de Morlaix. 9-13-73

Sept. 14, 1973. "Caves" off Ile Callot, Baie de Morlaix
We were taken by boat to Ile Callot; however, I have labelled specimens Ile Callot. 9-14-73

Sept. 15, 1973 Rostiniec low tide 9-15-73

Sept. 18, Ile Louet. Dredging between this island & rock to south. A very narrow channel.

Intertidal ~~walk~~ walks #1 & #2 refer to Sept. 13 & 14, 1973, field trips. Also Baie de Morlaix refers to these 2 days of collecting

The blue Bowerbankia bridge where stand the blue boats.

Sept. 14, 1973
tides were about 1.1, algae zone that we were on would be covered 2-3 metres.

Hemalun

Prepared hot

hematein . . . 0.2g.

alum de potassium . . . 5g

$K_2SO_4(NH_4)_2SO_4$

distilled water 100ml

Boil, cool, filter

add; acetic acid 2ml.

Bulbous alga — Saccorhiza

Codium (deadman's fingers) w. ctenostome

Fucus serratus

Anastomosing potato chip — Hippodiplosia foliacea

How did A. B. Hastings make measurements on G.B.R. material?

Hemalum

Prepared hot

hemateine . . . 0.2g.

alum de potassium . . . 5g

$K_2SO_4(NH_4)_2SO_4$

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Harmer, S.F. 1891.
Quart. Jour. Micr. Sc. 32: 127-181.
2 D Pl.

Busk, G.C. 1884. A synonymic
Catalogue of the Recent Marine
Bryozoa, including fossil synonyms.
London. 352 p.

Johnston, J. 1847. A history of the
British Zoophytes. 2nd edit.
London

Berg, F. 1931. Bryozoa. Die
Tierwelt ~~[illegible]~~ Deutschlands
17: 25-142.

Harmer, S.F. 1898
On the development of
Tubulipora & some British &
northern species of this genus.
Quart. Jour. Micr. Sci. 41: 73-158.

Pore plates — wide canal in funiculus stains pink.

Electron Microscope Photographs

1. Tubular appearance of funicular strands; like magnified Golgi app.
2. Collagen (starry)
3. Epithelial cell — non descript, exploded & granular "broken" wall
4. Peritoneal cell — nondescript.
5. Double layer of cuticle between 2 zooecia [this is important to note that G.L. has photographs of this.]
6. Wall structure — cuticle is smooth; calcite is granular
   ? depositing calcite is smooth

Mounts in Canada Balsam will keep if kept in a cool place.

Pore plate — em. photographs

H-cell in pore plates.

Microvilli around pore plates.

Nerve cell – has distinctive mitochondria & peculiar incomplete tubules.

This cell ~~looks~~ has different aspects – it can be black or lighter colored depending on the stage of development.

Nerves

Striated muscle in parietal diaphragm muscle.

Haematoxylin stained Membranipora membranacea

there are 2 small glands between the opercular & diaphragm muscles in M. m. Spherular leucocytes lining the opercular lining; there is a doubling over of the lining.

Muscles – those attached to frontal wall and lateral wall — go to funiculus

inter epithelial & attached to cuticle

1) Schizoporella _____. Vestibular glands + a bacterial gland.

2) Bacterial glands associated with avicularium; central vicarious avicularium — Brella.

3) Single vestibular gland — Figularia. Vestibular gland comes off the diaphragm Bacterial gland (or bacterial organ) in funiculus. Vestibular gland secretes mucous + ∴ call it a gland; however, later G.L. differentiated bacteria "glands" because of mucins secreted

4) Vestibular gland + bacterial gland + spermatozoids + spermatozoites in Parmucellaria. Vestibular gland is contorted. Bacterial gland below vestibular gland.

5) Bugula — spermatozoids + eggs in

6) Beania mirabilis — bacterial gland in funiculus. Stages of bacterial encystment in lumen. Slide shows changes in cell + in bacteria as encystment progresses — these cysts have been mistaken for ovaria. B. mirabilis is very difficult to get in dredgings + v. difficult to stain + fix

Fluatra has large distinctive Hillet? cells but G.L. is not able to fix this species.

For fixation - fix with 2.5% glutaraldehyde & cacodylate or gutaraldehyde (2.5%) with Sorensen's buffer at pH6 to pH7.2. It is important that the osmolarity be that of the local seawater.

G.L. will only work with anaseans because decalcification causes so much shrinkage that you can't sort out the wrinkled shrunk layers from true structures.

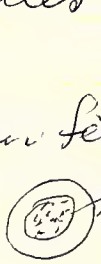
Wall muscle is continuous from the operculum in to the tentacles in

Muscles on cardia attach to basal wall.

Wednesday

Funiculus attaches to caecum but this can only be seen from the dorsal side.

Bugula "collar" (area of diaphragm).
Brown bodies — are they discarded.

4) Callopora (en fère) various stages of egg development  double membrane

5) Smittina — morula stage in ovicell. Also 4-, 8- & 16, & morula in internal sac.
Radial cleavage (check this — the cells showed equal cleavage at top & bottom — whatever that is.) Noted pseudopores which have at least 8 deeply stained nuclei.
Slide stained Haemalun with green

6) Again a warning against using trichloracetic acid for stained material

7 tentacles narrower — contracted sheath

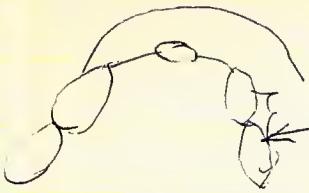
Slides examined (contd.)

1) Bacterial gland attached to brown body. Bicellaria _____. In this species the polypide is not regenerated. The polypide lives 3 weeks & has rapid growth resulting in extensive colonies.

Bacteria have no nuclei &. ∴ generally appear colourless & naturally not taking any nuclear stain. You need to isolate bacteria to establish if ~~the~~ it is bacterial gland using bacterial squashes.

2.) Flustra. Regeneration of polypides. As one moves to proximal part of colony you find ~~3 sets of regenerated~~ regeneration to 3rd regenerated polypide & 2 sets of brown bodies in the zooid. Also note sperm & eggs.

3) Spermatozoa develop on walls of Umbonula. Grellet (who worked in CNRS lab, 1952-60.) studied spermatid development but G.L. uncertain where information was published. Spermatozoa are generally on lateral walls in funiculus.



← 2-lobed vesicular gland.

3) cellepores are v. contorted; there is a progressional systematic arrangement; — see G.L. publication.

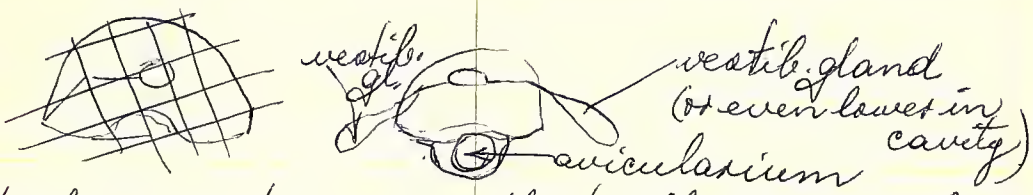
Rubbermaid for microscope pad.

A. Groat hematoxylin — spines develop proximally in Membranipora membranacea. As polypide buds some epithelial cells are elevated and any elevated epithelial cells are places of budding ∴ spines develop.

8-10 zooecia / day. Average of 5 zooecia per day with several levels of developing polypides.

Slides examined in Paris (contd.)

1) Vestibular glands in Schizoporella are way up near diaphragm & "appear" to come off operculum.



Nuclei are stained so that cells of funiculus are clear.

2) Sometimes brown body in coelom consists of dispersed material which is NOT encysted

3) Anus always links onto vestibule at dorsal side — check this.

4) Mucronella — hypostegial cheilostome with pore plates elongated into tubes (walls look as though they have parted in places). V. thick colony — ∴ whole mount is v. difficult to analyse.

5) Schizoporella trispinosa — 3 spines above operculum. Except for green stain of outer ring, I would also think of these as pseudopores (also sort out spines and eggs!!).

Bacterial glands in giant avicularium.

Vestibular glands show a progression from 1) simple single lobe; to

See over → 2) 2-lobed vesicular gland; to

Slides examined in Paris

Smittina _____. Looked at various stages of egg development from initial cleavage stages, follicle stages, 4-cell, 8-cell, 16-cell + also 2-cell. In ovicell (O) initial "invagination" differentiation of "internal sac" Note no digestive tract etc. in 3 "genera" with cyphonaute larvae. After "internal sac" forms this cements to become the basal wall.

In morula stage you could see nucleation of cells from crown and still some syncitial?? cells (ie. cells w. nuclei but not separate wall membrane)

Eggs do not occur in ovaries. They ~~are~~ may be massed together in funiculus & display characteristic follicle stage.

In looking at larvae in ovicell, the pseudopores (in anascans these pores are called) are aligned around the ovicell & show deeply stained nuclei, ca 8 — this is distinctive of pseudopores. See Cyclostomata, pores likewise in this group have a distinctive stain.

Fix in field in sea water as you collect. Use Aqueous Bouin. Leave in aqueous bouin (which also decalcifies) for 1 or 2 days (maybe only 1 day if species is not heavily calcified). Then overnight in 70% ethyl alcohol. Then stain:

Stain could be: 1) Alumnate carmine
2) Groat haematoxylin
3) Haemalun masson

Then dehydrate & mount in canada balsam.

If using a neutral fixative which does not decalcify use 10% ammonium citrate — this is for histologic stains. <u>AVOID</u> trichloracetic acid for histology of any kind as this destroys the tissues. (it may decalcify but it destroys everything else). Also AVOID EDTA because you will have the same problems.

When we looked at spermatids in colony G.L. showed me a zooecium wh. had spermatids + ~~ovaries~~ eggs together. She says there is intracolony fertiliz. as well as crossfertiliz. She would view dev. of sperm and eggs as occurring when colony has reached a certain size i.e. certain stage of maturation i.e. there is a certain phase in ontogenetic development when sperm + eggs develop — there are no "ovaries" or "testis" in bryozoa, this occurs in coelom

Wed. Oct. 10.

Looked at slides w. vestibular glands + bacterial glands. G.L. has published on this. I agree ~~that~~ her public she identified ~~identifying~~ some of the bacteria as sporozoites but now admits they are bacteria. She tried to isolate bacteria in vivo + identify them but no one has so far managed to cultivate symbiotic bacteria. The bacteria differ in form from sp. to another sp. We looked at boxes of prints of bacteria in various spp.

Slide 1. Large paired vestibular + a large single bacterial gland in Schizoporella

These are extraord. large.

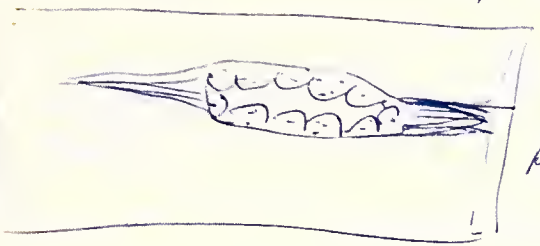
Slide 2.

In examining stained (hemalun) slide of Electra loaned by G.L. from her Paris collection, I tried to determine attachment of funiculus to digestive tract. It clearly attaches to caecum & stomach & G.L. later indicated that digestive tract is "enmeshed" in funiculus all over.

Anus opens within lophophore! This is a poor description of the situation Anus opens into tentacle sheath.

(39)

Views of a budding zooid on Membranipora membranacea grown on slide. : looked at basal side. M.m. had been growing on slide for 3 days.



Row 4

Oct. 1, 1973

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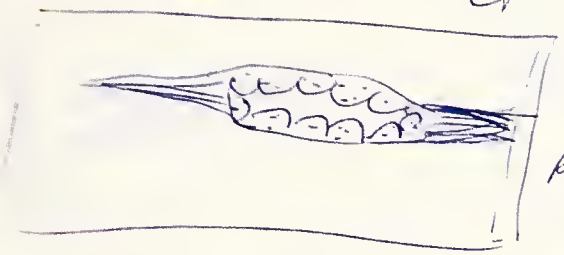
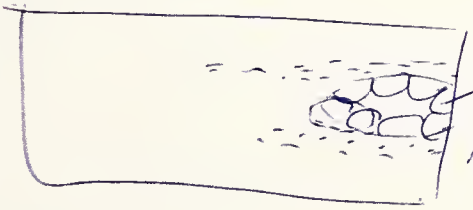


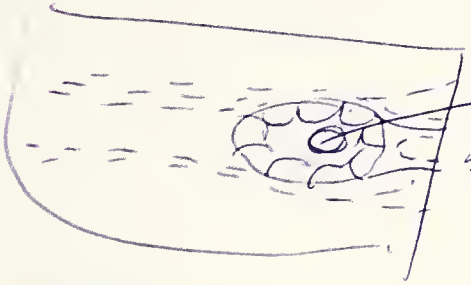
Row 4

Oct. 1, 1973



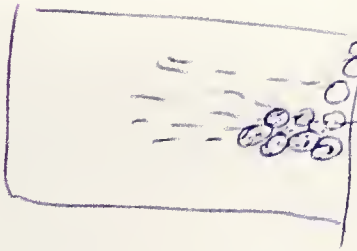
tentacles

Row 3 from dist. edge.



stomach or anus sing

row 3 from dist. edg



row 2

granular - aphenular leucocytes.

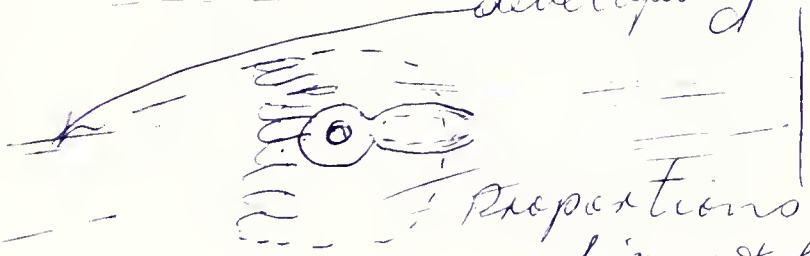
In most instances where "granular" mass of dense cells at proximal wall - other aphenular leucocytes ?"hide" the funiculus

Row ~~B~~ 5 from distal end.

developing tentacular sheath.

Row ~~B~~ 5 from distal end.

developing tentacular sheath.



Proportions incorrect tentacle are a diam. & length too large.

When tentacles are developing & anus is beginning to appear funicular strands near gonocial wall appear more dense — just a feature of slide???

Tubularia grows rapidly & therefore can get it on the glass slides. Hydra grows v. slowly & it is v. difficult to get it transferred from thin red alga onto glass slide

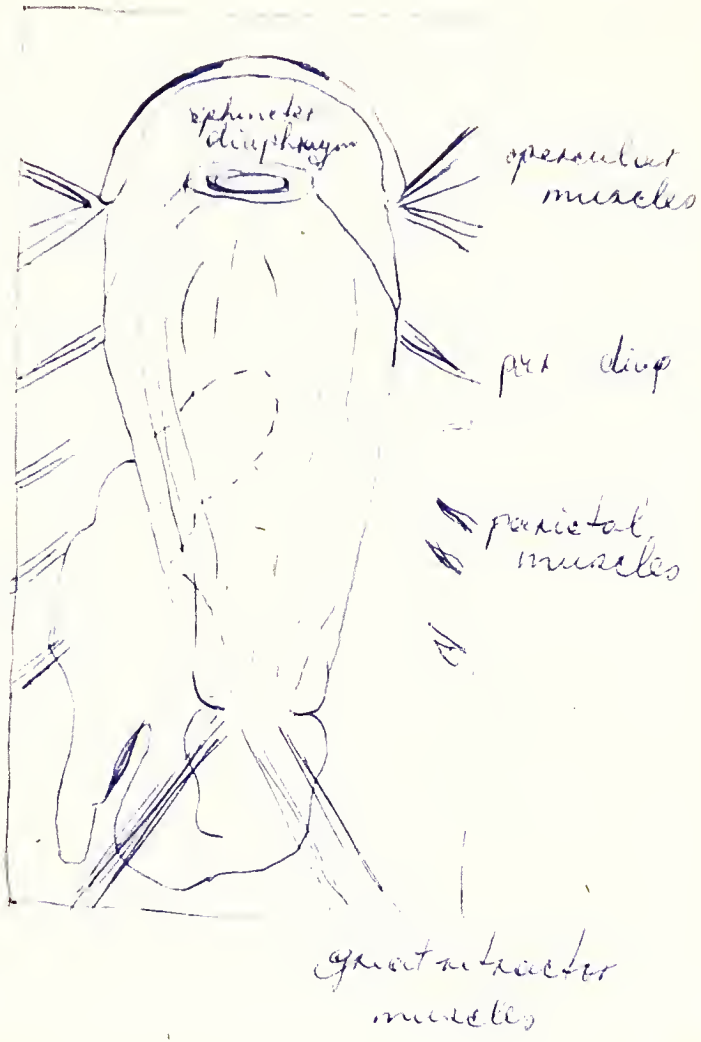
Oct. 1.

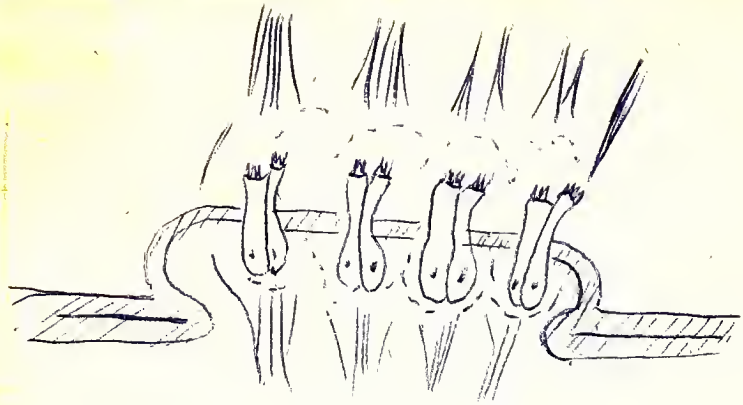
Vestibular glands – I had looked at K's slides the night before & so asked about v.g.

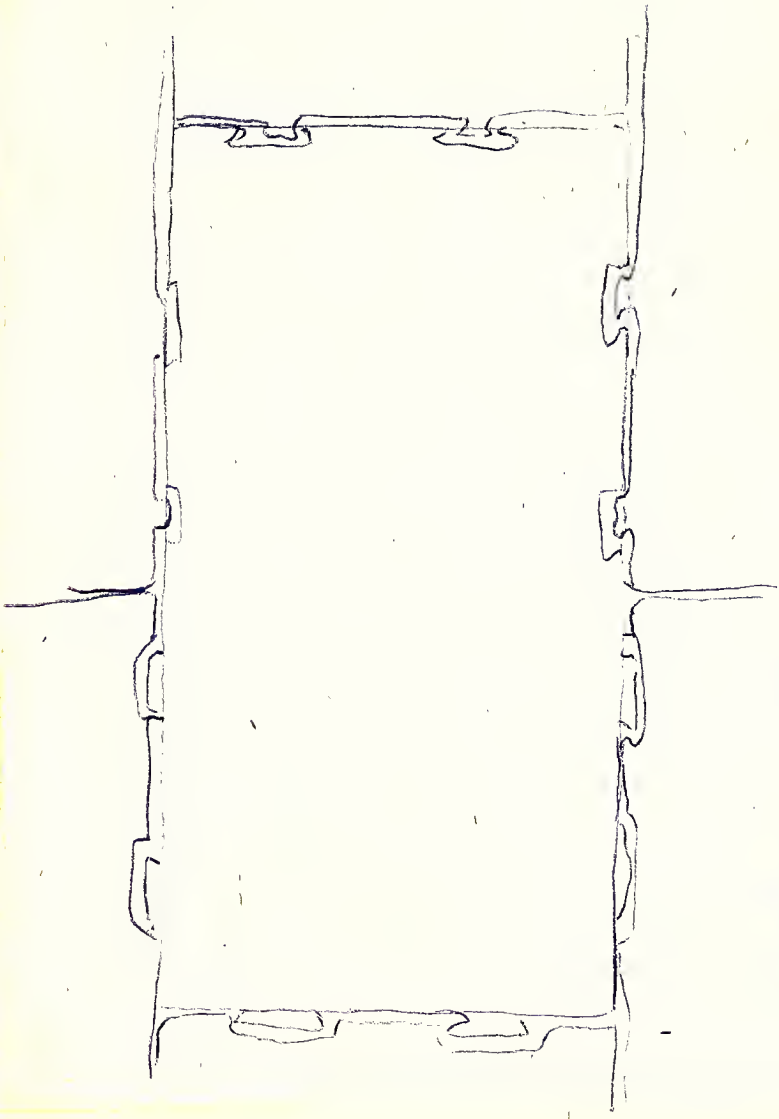
G.L. V.g. are present in most ascophorans – not present in anascans. V.g. are char. at family level; they are commonly infested by bacteria

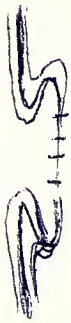
V.g. can be seen in whole mounts & up to oil immersion in light microscopy – you might need 200 slides to determine orientation of v.g.

Question about "replenishment" of seasonal bry. Acc. to G.L. larvae may develop from 1 zooid or 1 zooid may start to bud again. Larvae go deeper in water in Fall & Winter eg. Bugula from Nov.–Feb. is v. low in glycogen & ∴ larvae cannot develop. In Feb. glycogen builds up & larvae begins to develop. Larvae could go down to 30m. It is the upper level which is restrictive in occurrence of species


sphincter diaphragm
opercular muscles
part diap
parietal muscles
great retractor muscles

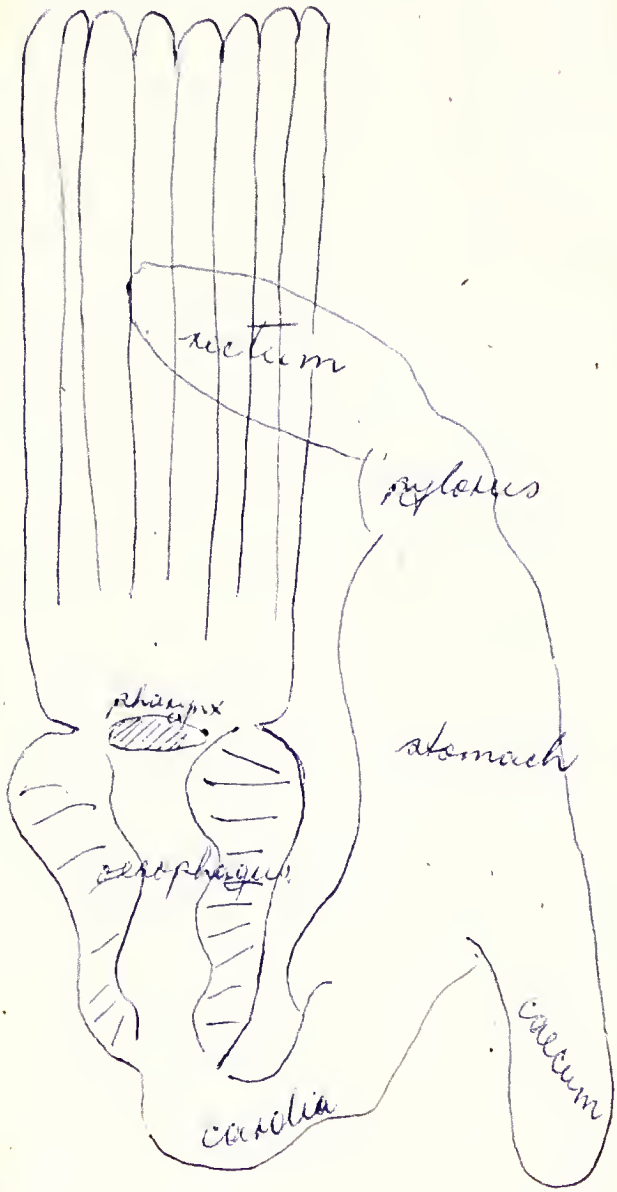






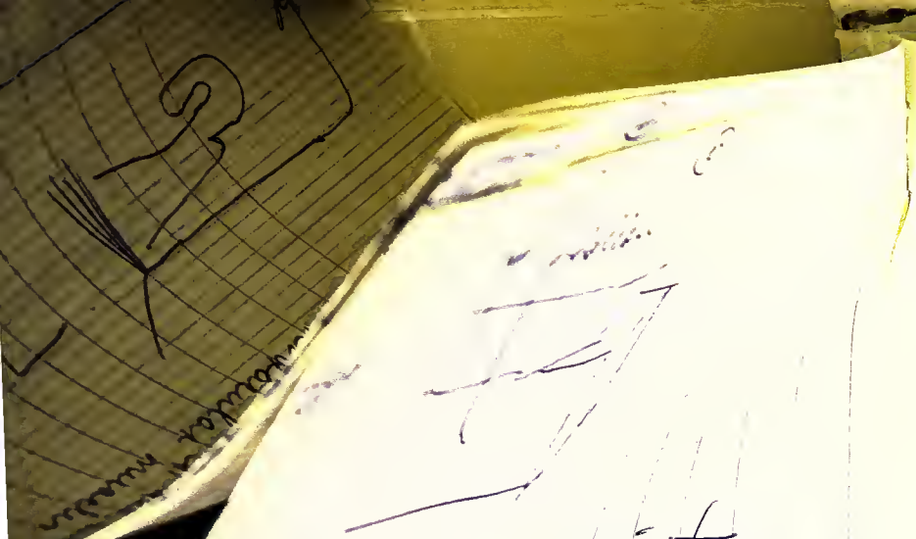
pour plate

(ce)



2-part polypide

Avicularium on stained Bugula

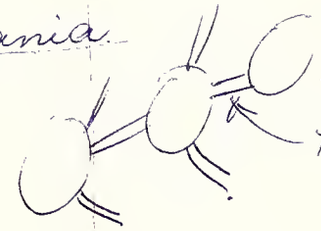


...ads or imagination of
...lumceno ??
— expansion of wall
— kenozooid
...ds articulated spine

...horizontal wall budding — does
...belong to the considerations
G. thinks so.

~~Woollacott Soule~~

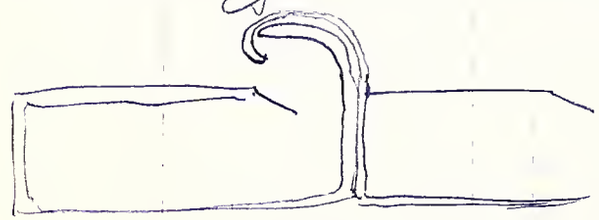
Beania



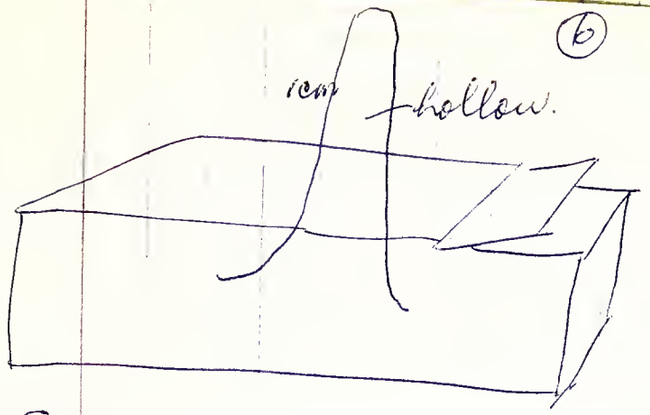
these are kenozooids

Microporella

Then to Ovicells see Woollacott & Soule theories
the various positions of ovicell.
(Check monograph on Ovicells — is it Harmer?)
?? Could these be kenozooids or spines ??
Woollacott (in press) considers ovicell ~~co~~ develops
from double wall fold from zooid w.
ovicell + zooid distal to this prev. zooid



↓to something like this.



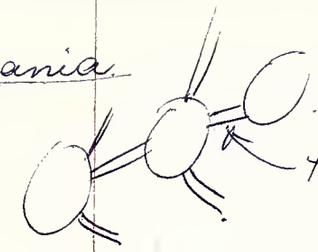
~~Cenozoids~~ Spines are buds or invagination of wall — ∴ call them ceno ?

3 types of spines — expansion of wall
— kenozooid
(no funicular strands in spine)
— articulated spine
eg. Microporella pallasiana
~~a~~ b).

See Banta frontal wall budding — does this phenomenon belong to the considerations outlined here. G. thinks so.

~~Woollacott, Soule~~

Beania



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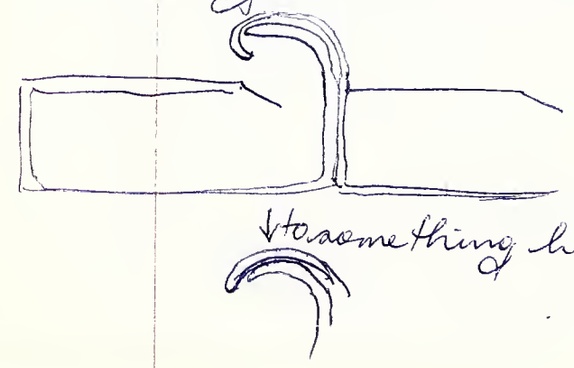


↓to something like this.

{ Semicellaxids are char. by vibracula
{ (subfam. Bugulidae)
even in some anascans
Conescharellidae.

Theory regarding occurrence of Avic. + Vibracula
2 theories

1) avicular dev. due to prevention of lateral budding

2) Many buds + lat. ones are reduced or suppressed

3) Maybe lateral budding occurring after calcificat. + then polypide is reduced in opportunities

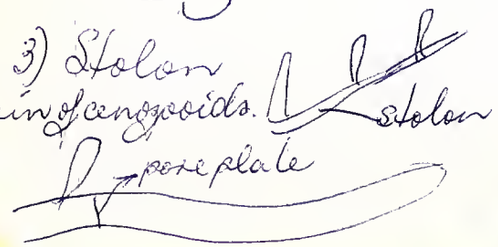
Cenozooid.

1) no polypide, just wall.

2) struct. squeezed. betw. autozooecia

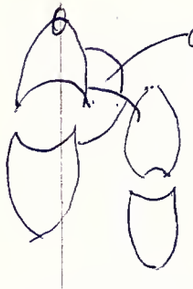
has funiculus, pore plates

3) Stolon chain of cenozooids.



modif. Membranipora
lateral buds became stolon in G's expt.

Chorizophora bronzhanti.



Now dietellae Halopoma
cheiloporids

double wall betw. dietella + d. w. betw. d. + autozoid

Is dietellae a pore plate or a cenozoid.

Is pore plate a bud?

Radicells.

← radicells are not nec. prerequisite

Bugula + Semicellaria radicells rootlets.

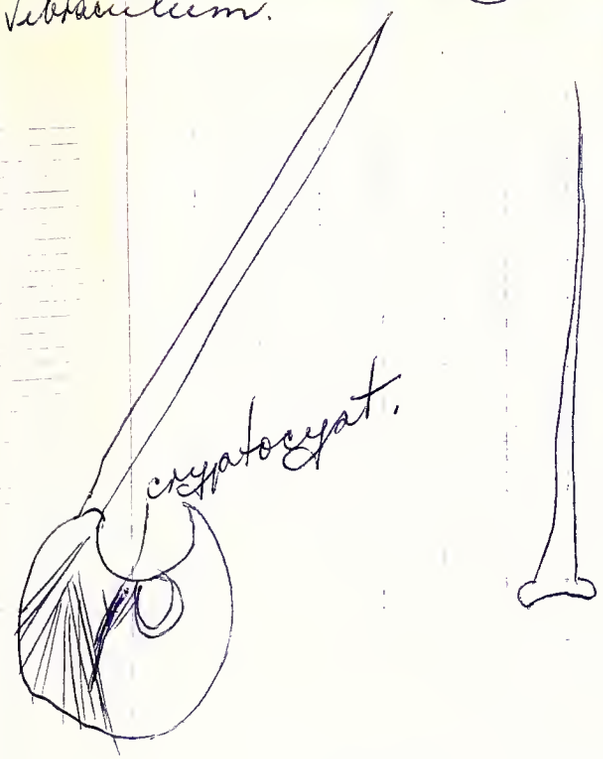
(Rootlets have funiculus & think)

Tower cell in aging colonies under certain conditions Membranipora phenomenon

pte.

vibraculum.

(2)

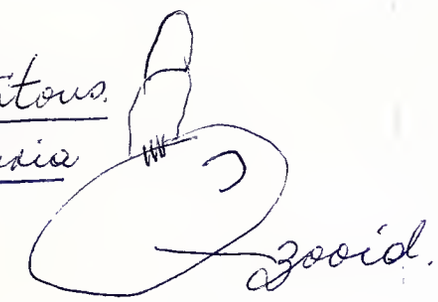


Various types of avicularia. See Silen's paper (3)

sessile avic. has kenozooid of articulation

eg Bugula

Adventitious Avicularia



Vicarious avicularium

Avicularium

cells of epithelium are ciliated

polypide bud reduced

Not a sensory cell has some innervation

no tentacles

cells secrete a mucous sub. at one time led people to think avicularia were sensory cells

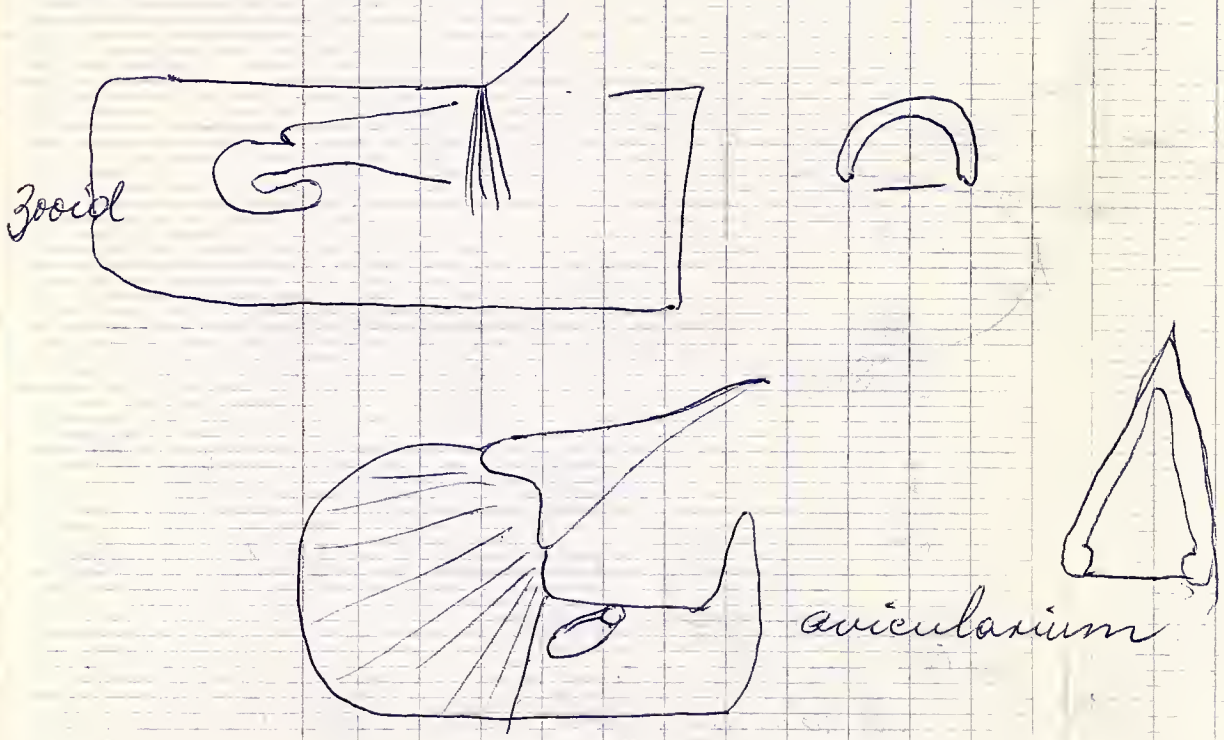
(33) Heterozooids then [Later Cenozooids] Sept. 17

Buds wh. don't function as autozooids & wh. have something left of polypide.

Cenozooids in wh. no polypide is present & can come from lat. or linear budding.

Heterozooids lat. or front. budding wh. are adventitious "secondary" — char. by
- avicularium or vibraculum
- {operculum & operculat. muscles hypertrophied
- {mod. operculum in avic. as mandibles
- {setae of vibracula are modif. operculum??

Avic. is reduced polypide w. reduced muscles eg. reduced tentacular muscles.



[Note afterwards — Ideas running through here & arrangement is Hincks, 1880, Brit. Mar. Polyzoa]

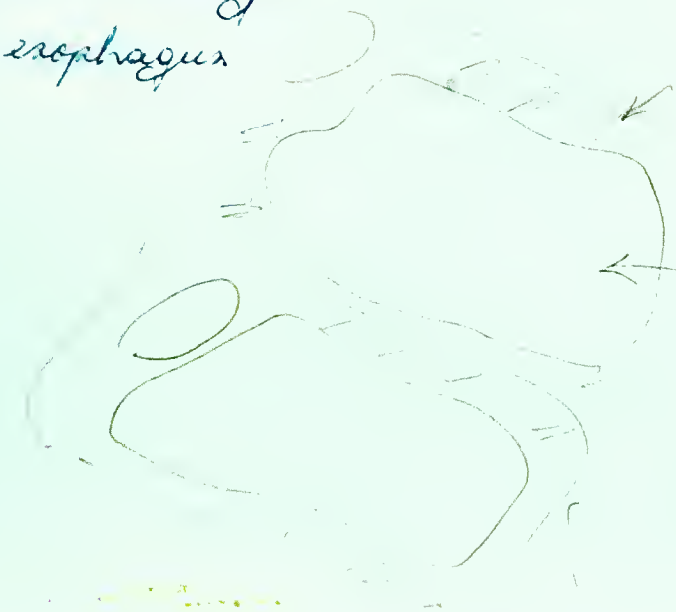
E.M. work – Phyllis Broadbury told Rumanian (31) ciliate workers that the one way to hold e.m. tissue (protozoans) after glutaraldehyde fixation was to put them in 6% sucrose solutn with cacodylate as a buffer & then in refrigerator

G.L. said one needed a hard epon for bry – but there was the difficulty of penetration w. all e.m. resins in bry.

G.L. is going to try Spurr

Esophagus - neutral red staining
[illegible] stained [illegible]

Cells lining
the esophagus

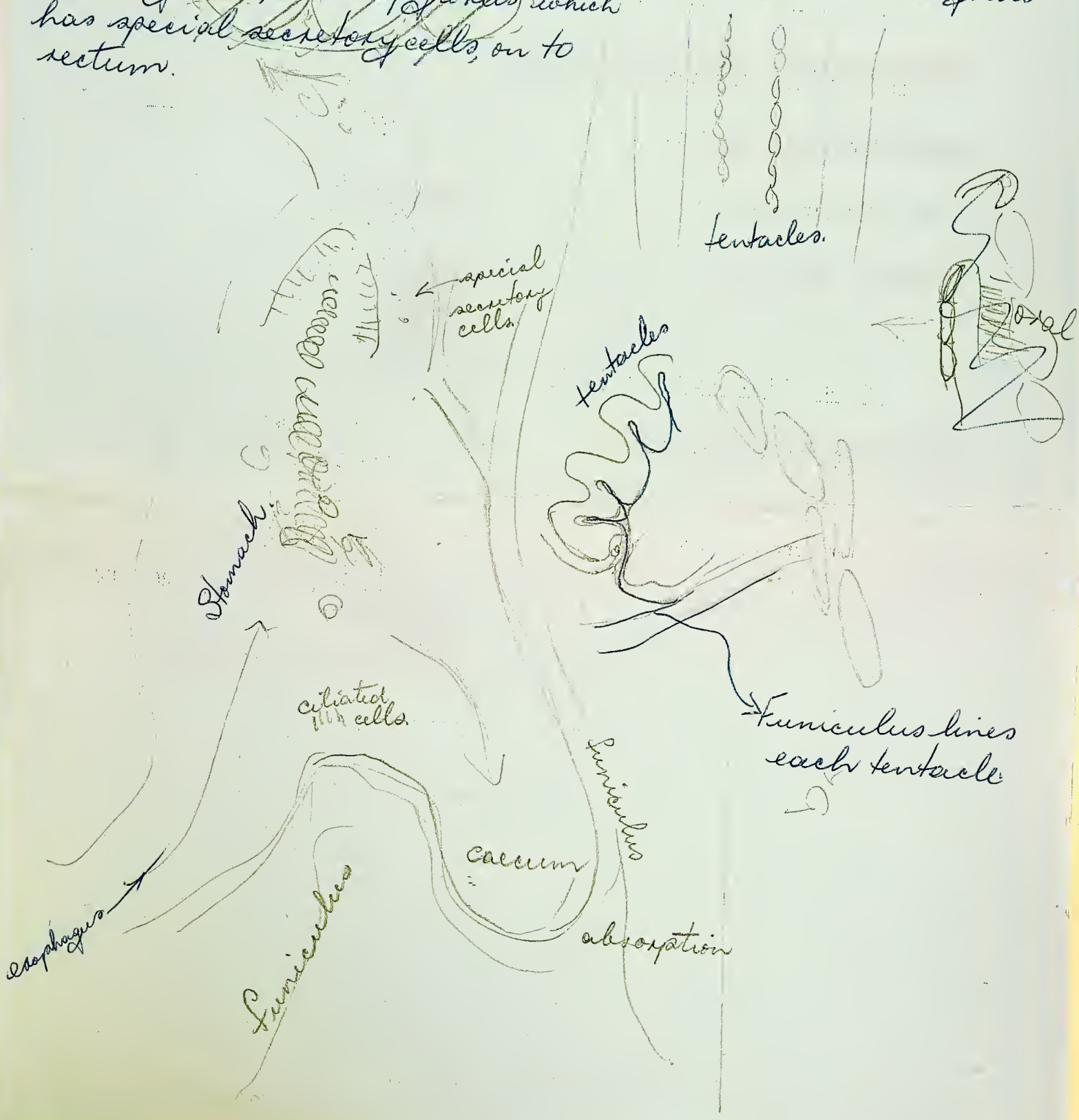


muscles.

[illegible] cells
v. special to esophagus

[illegible]

Food is ground in esophagus, passes into stomach where various secretions + twisting of material takes place, then to caecum — major point of absorption — on to stomach again, where joins twirling mill, then to pylorus which has special secretory cells, on to rectum.



~~28~~ Staining. Monday, Sept
29) ~~Methylen~~ Cresyl blue
30x 4 aliquots from
solution
Ctenostome ( )
laminarian were O.K.
too long or too concen
Flustra.

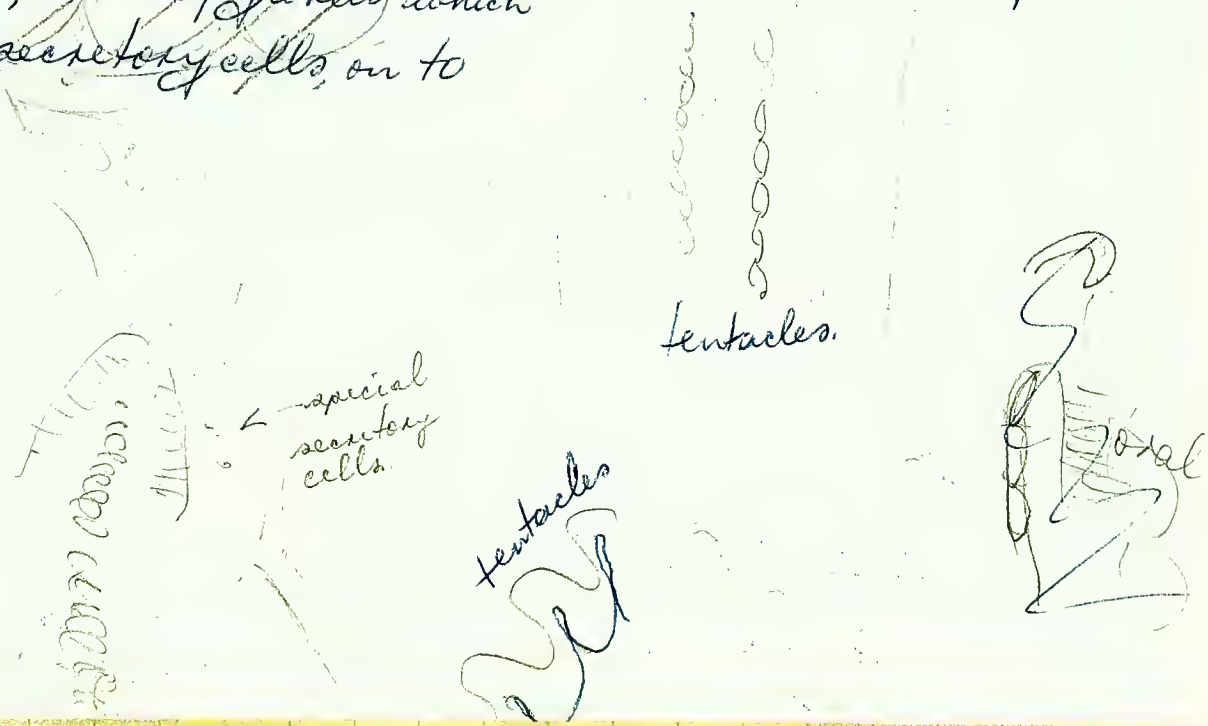
Typical basal bris
in wall of Flustra a
structure seems to
mouthings up tenta
membrane of tentacle

In Electra pilosa?
(~~vibracula~~) stains bl
spines do not stain bl
stomach has 3-4 bl
structures with extens
digestive tract. Tenta
stained even though
to lower ⅓ of zooecium

Ventral Rad.

Material in 3-3½
Similar results to cres
stain taken up by m
Bugula with ramifyi
frontal wall. -2-0

Food is ground in esophagus, passes into stomach where various secretions + twisting of material takes place; then to caecum — major point of absorption — on to stomach again, where joins twirling mill, then to pylorus which has special secretory cells, on to rectum.



Staining. Monday, Sept. 17. Observations in Vab.
29) ~~Cretaceous~~ Cresyl blue brilliant
30 or 14 aliquots from pipette of standard solution
Cenostoma ( ) & Electra ~~vilosa~~ pilosa on lamination were O.K. after 2½ hrs. This was too long or too concentrated for Bugula & Flustra.

Typical local birefringence of funiculus in wall of Flustra stood out. This same structure seems to longitudinal nourishing up tentacle sheath (or is it membrane of tentacle sheath

In Electra ~~vilosa~~ pilosa? the low median proximal spine (~~vilosula~~) stains blue whereas the short spines do not stain blue. (In E. v. in Cenostom stomach has 3-4 large clear spherical structures wh. extend across diameter of digestive tract. Tentacular sheath clearly stained even though tentacles retracted to lower ⅓ of zooecium

Neutral Red.

Material in 2-3½ hrs wh was far too long. Similar results to cresyl blue in amount of stain taken up by various species & genera. Bugula with ramifying "spines" around frontal wall. ~~[illegible]~~ -2 or 3-fingered arrangement.

56

(28) Recollections – hope sequence is correct but may be wrong
Film. Growth of Membranipora membranacea
prepared from G. Lutaud thesis, used in
classes. Photog. by Mr. Panelve who is
doing a whole series.

Budding v. rapid & colony edge grows
v. quickly. Longer growing edges – do they
produce longer zooecia? Yes –
G. explained development of polypide from
proximal wall & said there is no way
of separating polypide & cystid. Initial
"mass" of polypide appears at prox. end as
an area pulling away from prox. wall
this grows & forms area w. diaphragms
from wh. tentacular sheath develops.
The funiculus starts out as single strand
& gradually separates into several strands
likewise muscles start to appear. Then
rapid dev. of digestive tract w. anus
being one of first parts to appear.
Polypide ultimately breaks a membrane
to extrude tentacles from operculum
wh. appears rapidly after diaphragm
development due. wh. nervous system
starts to develop.

Note: white globules (polysaccharides) at budding
edge of colony

(27)

Cyclostome on bulbous area of "spiny" algae.

Tentacles have brown pigments in upper half to upper 2/3 – discrete pieces 6 to 8 pieces in tentacle. At first I thought they were concentrated on outer side of tentacle but at least at tip where tentacle tends to "claw in," brown pigment seemed to be in middle or inner edge.

Thin calcareous collars are perforated by whole series of pores at distal end of zooecium.

Escharoides coccinea on rootlets of alga

Stains. Rouge-Neutre, + Cresyl Blue.

1g in 100 cc distilled water (aqueau distilled) Let bry. stand for an hour (maybe 2 hrs); this will vary with species.

Rouge neutre (microbiol-physiol. product)
Bleu de Cresyl brilliant (Biohyon product)

Methylene Blue.

Crystals of m. b. saturate sea water. — cover solution with parafilm + place in oven + bring to 25°C. w. bry. in m. b. Oven is already on.

Caecum is gen. identified by constrictions both at distal & prox. ends

Spines wide at base & have many layers of cells making up cuticle.

Brown bodies & formation of new polypide — new polypide had "pouches" of intestine, stomach & rectum. "Pouches" might ~~suggest~~ are an aggregation of columnar epithelial cells so once you remember what they are, they are quite characteristic

On Sat. night, Genevieve & Gilbert Deroux talked about the seasonality of many of the bryozoans. G. has taken up Electra as she can get it year round. Membranipora seems with Fall equinox but this year is [Best tides are Spring & Fall equinoxes] not all abundant — too warm a year according to G.L.

The muscles of the operculum + diaphragm are on the tentacular sheath.

3 stains for whole mounts are haematein, carmine and haematoxyline.

Found a zooecium in Electra (always mounted so you saw the basal side) wh. had no polypide — something had happened so there was no differentiation but there were cells w. nuclei throughout wall. However, there was a pore plate that was differentiating — G.L. indicated that organism will always differentiate around pore plates even if polypide development is suppressed.

With methylene blue organism takes up m.b. then dies just as m.b. taken up in nerve. There are 3 processes (sequential)

1) 2) 3) oxidation

of m.b. so it shows up as blue stain

I looked at diaphragm in Eolectra wh. is v. diffuse in relaxed state when tentacles are withdrawn & I could see that it is part of vestibule wh. is part of ~~body~~ wall. Tentacle sheath likewise is a "soft" structure ident. only by red stain of nuclei so you have a net (& for diaphragm a clustering) of red-pink stained nuclei. Diaphragm has a canal thru it wh. is visible when have animal w. extended tentacles (G.L. would agree w. Wallace that ectoprocts are closer to Coelenterates & annelids than to present classif — I questioned her about other worms whereupon she indic. that Vermes was a better arrangement than the present splitting up into phyla.)

To relax bry. G.L. uses a few crystals of menthol in sea water tube. It takes at least an hour or longer

To mount slides dessiccate stained material & coverslip w. Canada Balsam wh. hardens w. age

Sat. Sept. 29 (22) (20)

Prepared slides made 10 yrs. ago using Hemalum stain. Nuclei stain red, muscle ~~blue~~ green, vesicular leucocytes ~~green~~ pink, spherular leucocytes bright green, polypidea red brown walls ~~hemalum blue~~ – this was for Electra ~~villosa~~ – & so colours would change for other species – however nuclei would always stain red. As you go through focus see nuclei in muscle. "Leucocytes" are simple polysaccharides acc. to G.L.

Acc. to G.L. muscle is striated, certainly all parts of digestive tract muscle are striated. After questioning she did perhaps wonder if sphincter muscle & tentacular sheath muscles may not be smooth. Again certainly all retractor muscles etc. are striated.

Polypide in Avicularium Bugula

This consists of 2 pouches – the atrium & stomadeum; polypide does not go beyond 1st stage of differentiation when it is in avicularium. Noted muscles in beak & also finer muscles opening operculum of beak.

8. Looked at setae (long whips) of vibracula & examined base of vibracula which looks just like an avicularium in outline.
9. We got to discussing diaphragm which goes across at base of tentacular sheath. G. could not remember if Bugula had a diaphragm.

G. did note that cteno-stome diaphragm seemed different to cheil. (am I remembering this detail correctly)
10. How many layers are there in peritoneum. G. says this is a real problem.
11. G. says there is epithelial peritoneum & peritoneal tissue lining digestive tract. This fact she is willing to use in identifying relationships of peritoneum.
12. The problem of chitinous joints in such spp. as Sertopellaria. The chitin has a laminate structure acc. to G.
13. Funiculus goes across pore plates (called diaphragm by Robin) Hence Chetham is suggesting diaphragm be dropped as a structure near "vestibule"

Sunday Sept. 16. Late afternoon

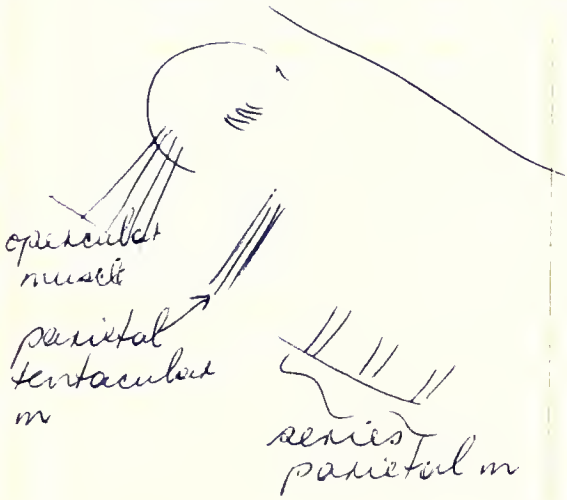
1) G. showed me spherular leucocytes – bunch of spherules wh. look like bunch of grapes
2) vesicular leucocytes – O large droplets also
3) pigments in outer body wall of Scrupocellaria
4. Earlier looked at funiculus (strand or bundle of strands) in Bugula. Funicular tissue aggregated around brown body in zooecium. G. thinks there may be 2 kinds of funicular tissue because 1 takes methylene blue readily (i.e. material around brown bodies); other strands perhaps more out in body wall of zooid.
5. Funicular tissue at growing bud tip forms mass of granular & strand tissue
6. Funicular tissue – v. fine granular (but without boundaries) – forms before polypide develops in zooid. Characteristic appearance according to G – we ~~at~~ looked at it in scope
7. ~~B~~ Disintegrating ~~brown~~ polypide appears like 3 brown bodies, piled one on top of the other. Char. again acc. to G.

G. again reiterated that you can't separate funiculus + polypide ie f. body wall + p. are all one animal + they function as much.

3. Bilaminate form of Flustra examined unstained. Funicular strands form plexus throughout wall + appear as highly birefringent beads — ∞∞∞ this feature is characteristic of all spp. of Flustra (generic character); they are lipids. (do not know what specifically)

4. G. says cresyn blue is a much better general stain for bry. We were looking at m. b. stained material because G. was after Hiller's plexus.

5. looked at muscles in Flustra



My diagram here is v. schematic & not nec. correct as I have quickly roughed it.

6. During brief notation re muscles G. said that Boardman felt muscles were arranged differently in cyclostomes

Sunday, Sept. 16. Staining in Lab. (18)

1. Methylene blue stain (do not know concentration) put Electra in m. b. in oven at temp. 25°C (at Woods Hole G. had m. b. at 30°C & got good results); leave in over 5 mins (ca 10 mins - here I am trying to recall the time G. put material in oven & then got it out.). Heat helps oxidation process wh. is what is involved in m. b. Place on slide, then coverslip, sea water w. pipette if nec. not more than 5-10 mins before animals are dead. G. finds if orient specimen under dissecting scope with bright mirror reflected reaction, the m. b. stain is better.

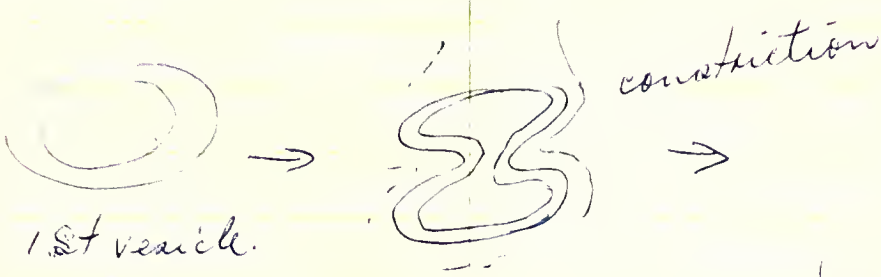
2. View species from dorsal side this is the only orientation by means of wh. you can see funiculus.

We viewed funiculus conc. around brown bodies — degenerating polypide provides nutrients (proteins etc.) wh. are discharged into funicular strands wh. are v. clear near brown bodies. There were cross canals closer to wall betw. funicular strands. G. commented that this was like a primitive circulation system.

(contd over)

Fri. Sept. 14.

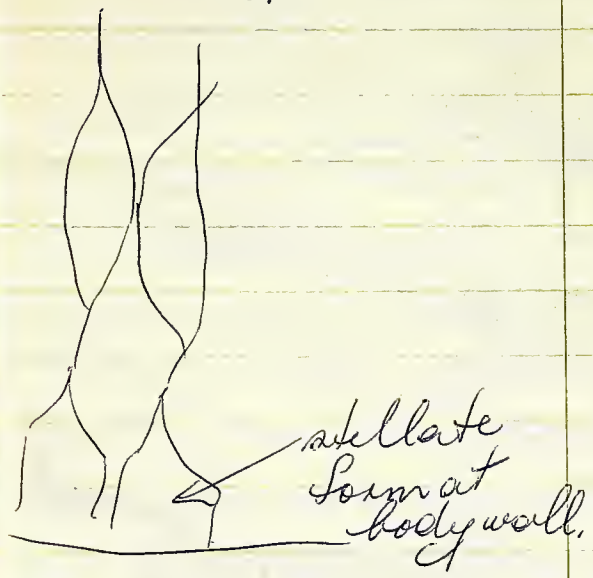
Polyoide vesicle - entoderm with its funiculus



ent. all around forms funiculus + forms canal (v. difficult to understand struct)
~~in~~ tentacles
entoderm [mesoderm] + funiculus strands of muscles

Side view series of 3 above stages

Form of peritoneum cells in funiculus & at body wall.



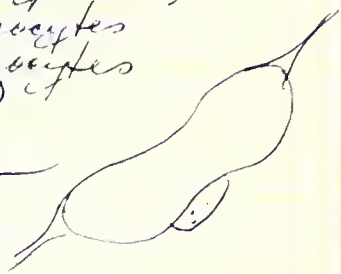
Frid. Sept. 14 -2-

Epithelial
collagen wh. gives rise to muscles
muscle

C. may not always be present but is nec. for fm muscle.

muscle.

Fumicles has 4 different kinds. Inclusion cells (lipids, pigments)
1) Vesic. leucocytes
3) Spher. leucocytes
4) P. (storage) cells.
Vesicular leucocyte segment

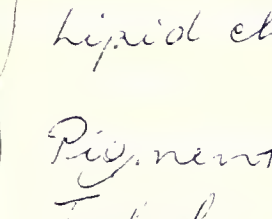


leucocyte spherule.

Lipid chains

Pigments.
Tubular cells.

also white cells wh. we see at budding edge.



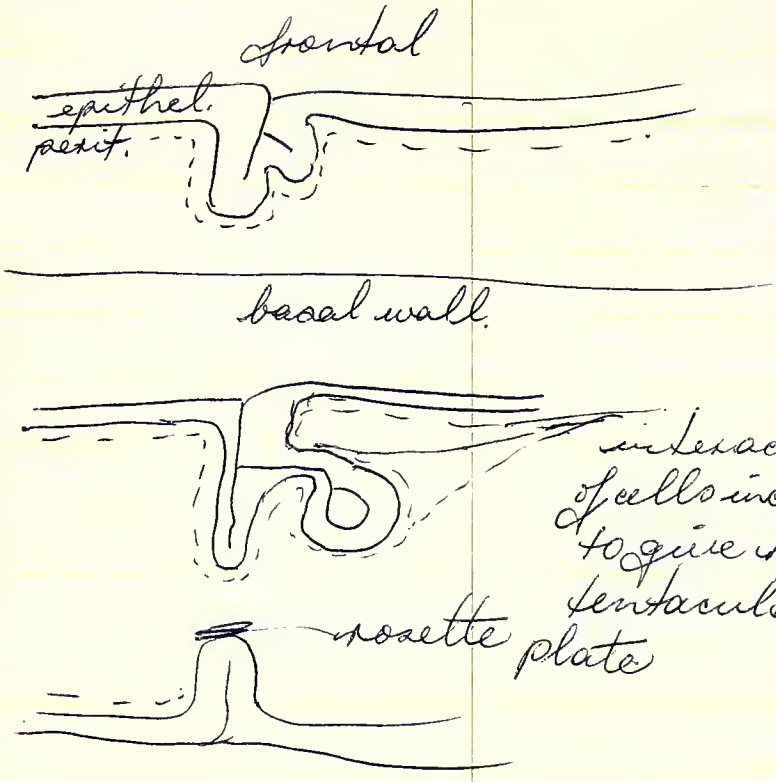
Peritoneum is mesoderm but this cannot be pressed.

Frida Field Lab Sept. 13

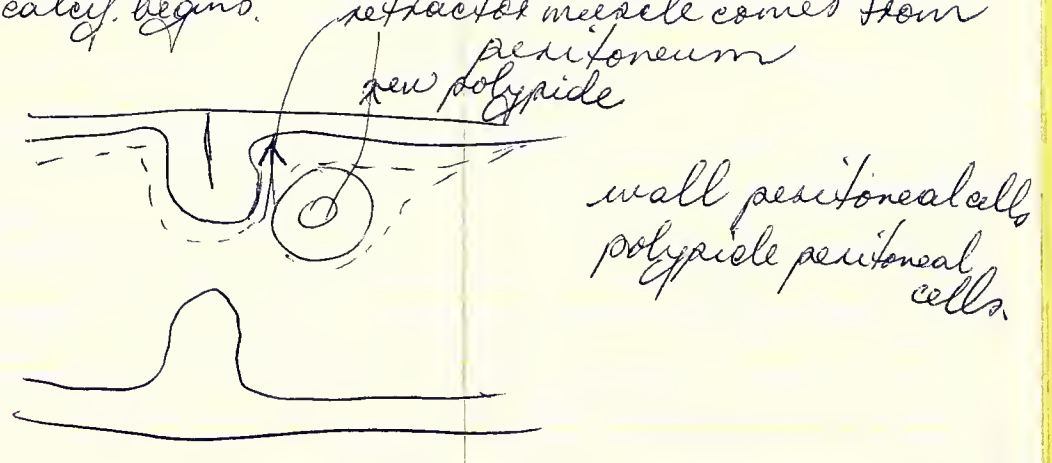
C. mentioned that lack of metallic ions i.e. Cu, prig. inds & oxygen respiration are the organs wh. should be investigated in frog tissue.

First polypide dev. from transverse septum.

(16)



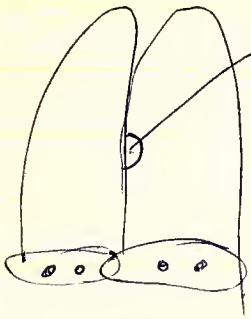
Morphogen. mov. must be completed before calcif. begins.



Sat. Sept. 15. Technique for getting budding bryozoan to move onto glass coverslip.

Use plastic peg clipped to laminarian + adjust cover slip so that is near tip of growing colony but not touching it

Cover top of tank to cut down light.



pore plate where embryonic cell is located in zooecium
pore plates develop precedes.
calcif. & involves solution of cuticle (see Silen & Banta)
& p. plates dev. where funiculus attaches to wall.

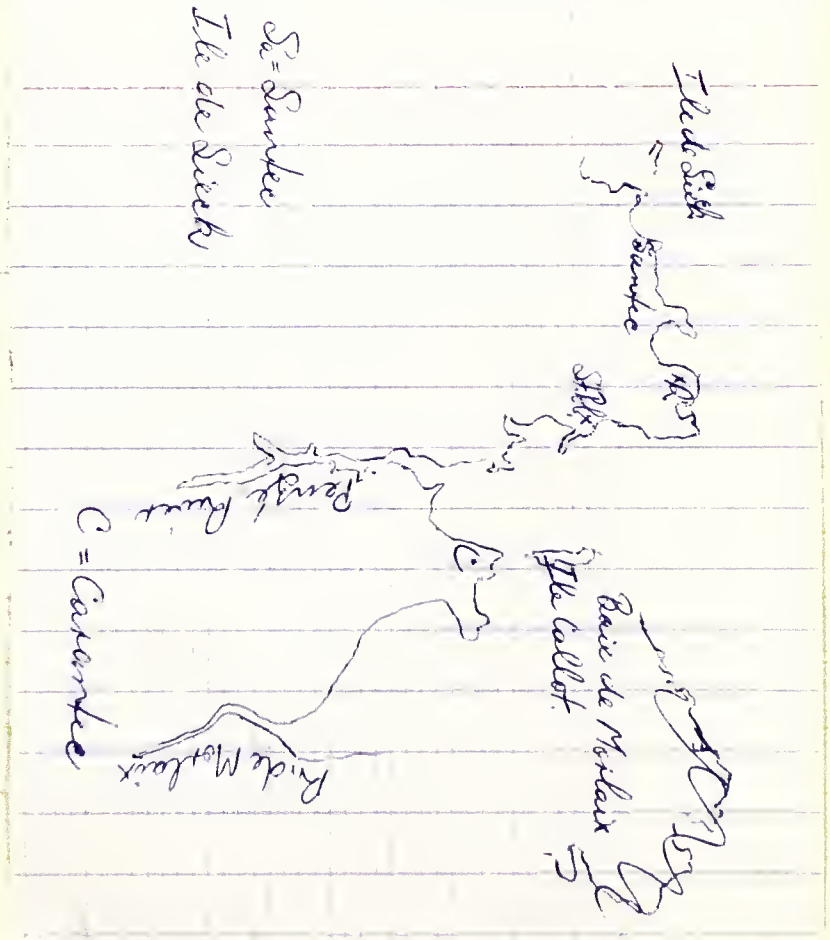
In cheilostomes
Peritoneum
Funiculus is dorsal; peritoneum is ventral
P & F. are both mesodermal & are essent. the same. In cyclostomes funiculus is centrally placed
Funiculus has all kinds of specialized cells — eg. glycogen (diffused glycogen in liquid form.
funicular strands release secretions around wh. cells will aggregate & involved in bro.

Longitudinal double wall.

Baie de Morlaix – anastomosing potato chip
{Hippalodipalosia foliacea

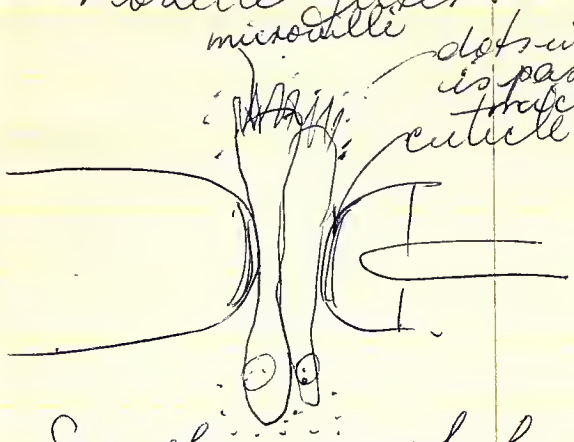
Thursday afternoon Sept. 20.

Examined collections from low intertidal from Rostivec. Abundance of Bugula spp. & algae w.



numerous ctenostomes & entoprocts. Entoprocts included Pedicellina cernua. Ctenostomes incl. Bowerbankia sp. This latter species was a form which left me still to work out how to separate Zoobotryon & Bowerbankia. Tentacles on entoprocts are held in position by an entirely different structure to ectoprocts

Rosette filter.



(14)

Seg. of mesench layers.
Squeezed into

Peritoneal cells are continuous w. funicular cells.

Monoserial colony forms have monoserial funicular strand
multiserial colonies have 2 funicular strand or 1 main strand wh ramifies to peritoneal cells at edge of cell.

($CaCO_3$ could be ontogenetic calcite or morphogenetic calcite.

Evolut of Peritoneum

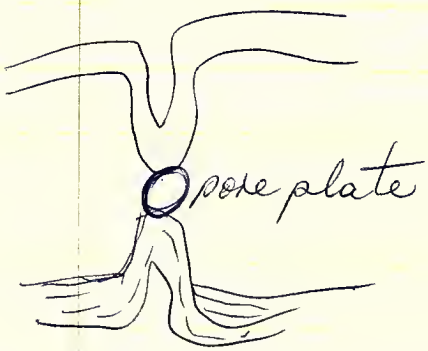
This is trophic organ of cell.

(13)

stellate peritoneal cells

elongate stellate peritoneal cells at tip of bud

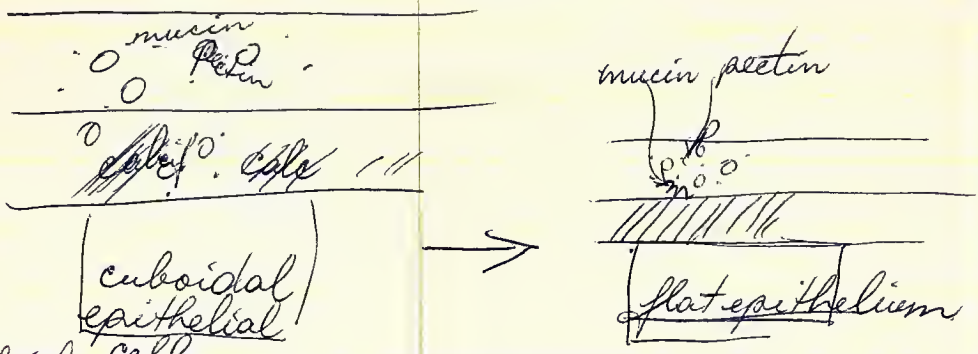
cells thicken & differentiaty these are part of dev. funicular strands.



squeezed cells of funicular strands → mesoderm cells of pore plates

Development of Epithelium (cont'd)
process of wall still flexible

(12)

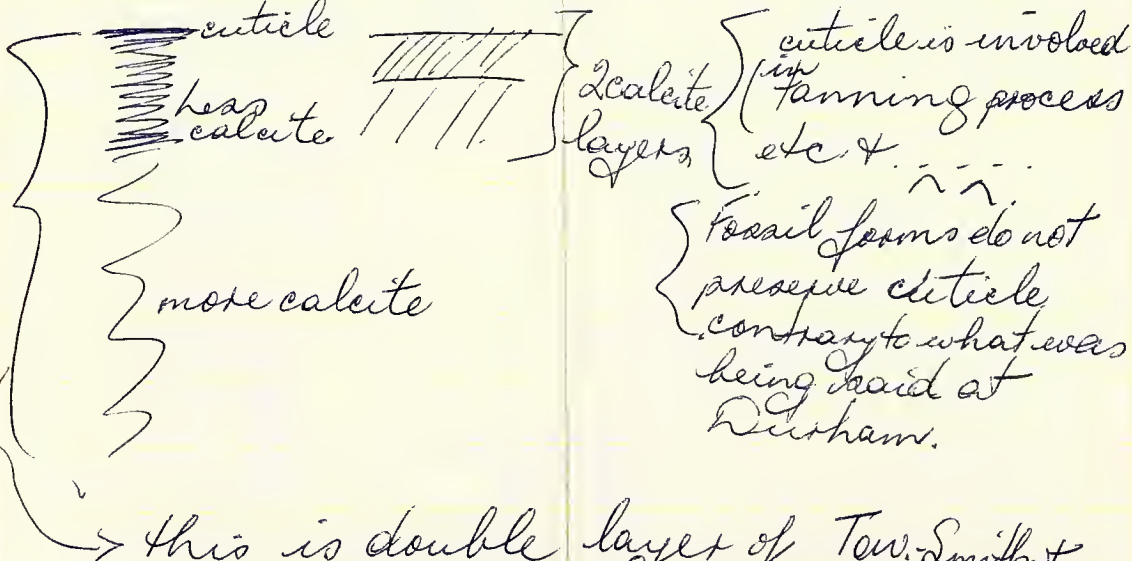


epithelial cell flattens & calcification continues.

G. thinks calcif. "inhibits" further dev. of cells [or if ossification continues there is no calcification.]

or further calcification leads to 2 calcified layers of paleontologists but this has nothing to do w. peritoneum.

In chil. more mucin in outer wall.



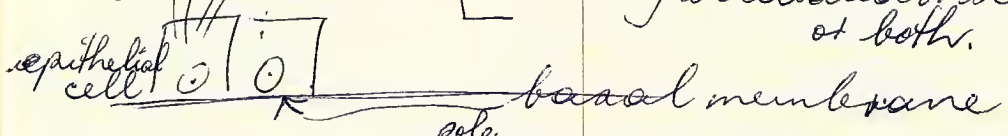
Perennial Bud.

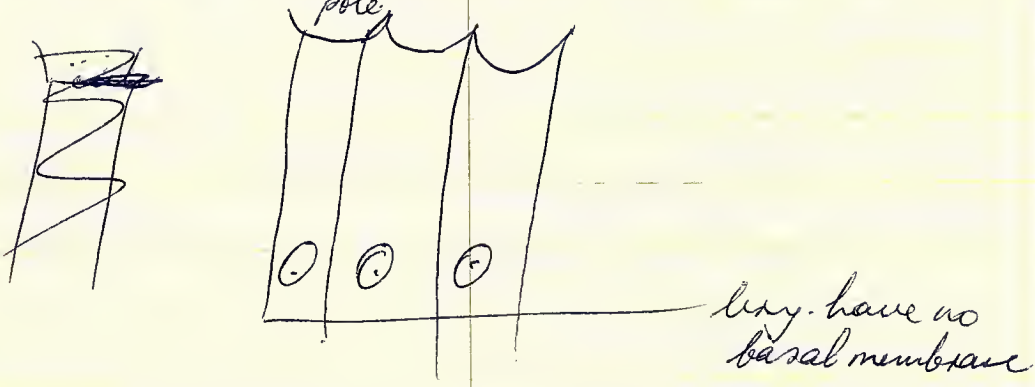
Embry. double layer (epith & peri.).
undiff. wall.

Calvet thought peritoneum was derived from epithelium but G. thinks 2 are separate w. diff. differentiation taking place in each.

Check this!! (O.K. when you season the out)
Undiff. cells at anterior end of zooecium
Differentiating cells at prox.

Generalisation about epithelial cells in organisms
Embryonic epithelial cell has pole & a basal membrane
ciliation secretion
cell may be ciliated or secretive or both.





secretion pole (wh. secretes ~~chitin~~ cuticle — mixt. of mucin & pectin
secretes mix. mucopolysaccharides & pectin then cell becomes cuboidal & mucin becomes dominant & calcif. begins after pectinisatn

VVWilliams.

Wed. morning

G. mentioned dimorphic forms of 1 sp. of bry. at Roscoff – you can see 2 forms have different colours in field but under microscope there is no apparent morph. difference

G. noted that hybridization is imp. in bry. but we virtually know nothing about the phenomenon

Thurs. Field Trip to St. Pol.

Bry. occur most abundantly where there is rock & sand – this provides sufficient "in-fauna" for food. Under ledges covered by algae.

Ctenostomes in upper intertidal area on algae.

Formalin fixation – stains go in v. well at cert. length of time after animal in formalin – perhaps 2 months ∴ have to check every 2 months or whatever interval.

(9)

Neutralize formalin w. $CaCO_3$ not Na_2CO_3 because of equilibrium of pH + saturation. ∴ use saturated $CaCO_3$. Na_2CO_3 results in continual alk. solution as material is fixed.

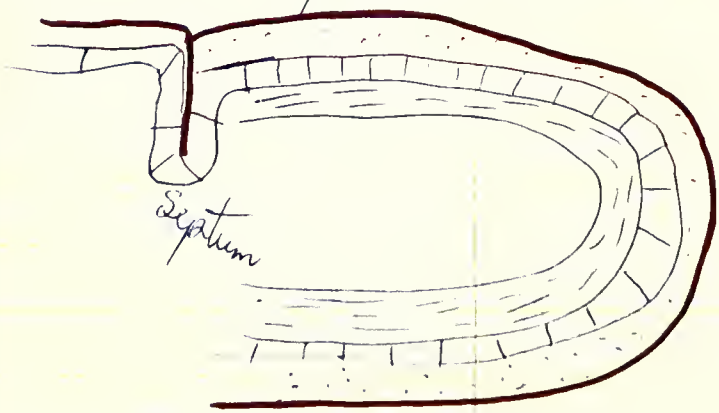
Picric acid in water is better than an alcohol base for bryozoans.

Acids fix cells – alkali breaks down cells as attacks proteins

Membranes fix well in ~~neutral~~ alcohol because of composition but nerve tissues do not – so depend what you are studying.

Ectoderm implies whole series of cells whereas epithelium specifies only 1 layer of special kind of cell.

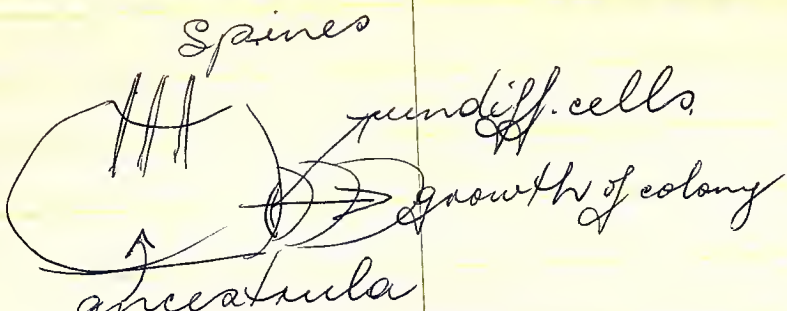
epithecar cuticle



Note.
Hyman, Fig. 110 p. 303 uses cuticle epidermis peritoneum

Great-grandfather.

Acc. to G. ectoderm is embryol. term implying embryol. relations.


Spines
undiff. cells
growth of colony
ancestrula

Epithel. cells always need a layer to grow on

Original young polypide from septal wall wh. becomes apex. wall of 3.
Regeneration of polypide from any wall or tentac. sheath, made of a zooecial possibly

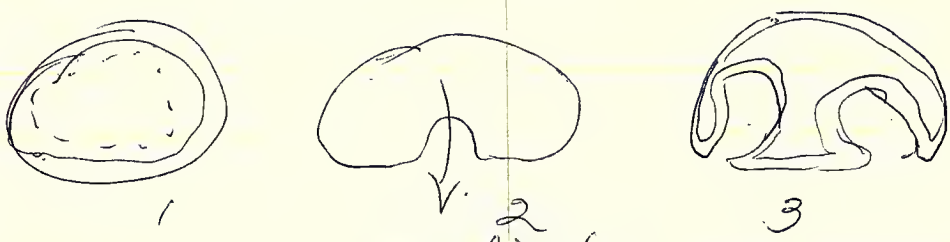
Ostrumoff, Pergens etc. argument about wh. one of them was seeing what — they were all correct but disagreeing w. each other in semantics

Cyphonaute 3 layers — ectoderm, mesod. & entoderm

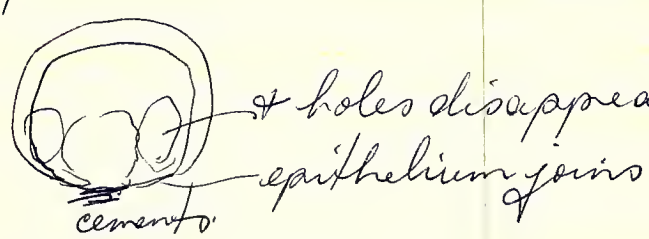
but in Membranipora wh. is a delayed form

these layers are not clear

--- mesoderm.



4



After stage 3, cementing of central part followed by epithelium of 2 lateral horns joining central part to form 2 circ. holes wh. then are enclosed & disappear.

Bez at Roscoff proliferated when oil covered the sea in English Channel - lack of light, lack of sediment.

Spine forms simply by upward projection of epithelial cells. The specificity of spines i.e. are there different kinds is a separate question in G's thinking

Epithelial cells always come together - you can't ~~part~~ keep them apart; they always have a binding action.

always some undiff. cells w/h. have potential as ontogenic cells to take off as growing tip.

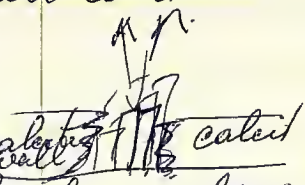
A bry. is a perennial animal w. potentiality

1. Differentiation — dedifferentiat. (5)

(can go both ways.

2. Cuboid → flattened cell.

3. Bb. in wall



always results in epithelial cells closing break + filling

4. Division of cell by lateral division

Intussusception

Is this a piling up or just accum. cells.

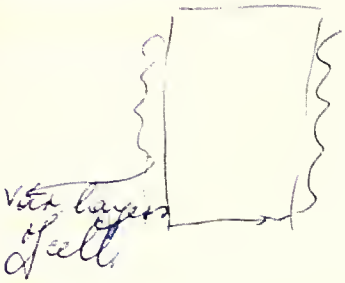
- G. views this as same but

Allan Cheetham views this as diff. process where cuticle is not involved.

Septal division

hongit — double long. walls

Cuboidal cell. → flattened cell.



Species to be found in Dredged Material from Ile Louet Buié de Morlaix

Aetea anguina	
Membranipora chelata	
Flustra foliacea	
Hemischaria Jhuxhoides	Hippothoa divaricata
Copidozoum curvirostre	Hippothoa hyalina
Cellaria sinuosa	Chorizopora ~~[illegible]~~ brongniartii
Beania mirabilis	
Cribrilina punctata	Harlopoma impressum
Cribrilaria radiata	Arthropoma cecili
Figularia figularis	Schizomavella auriculata

→

Keep bry. shaded (white slate) otherwise they lose their pigments but this of configurations. (3)

← configuration of tentacles.

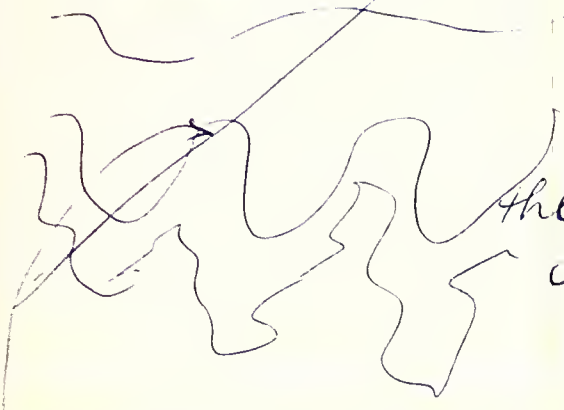
Ord. tentacle struct. not — McKinney

G. will bring Invert. recipe book back from Paris — these will still need to be modified for Bryozoa.

Gen. Anatomy - Hyman, Calvet, Lacaz (check)

you can't orient under e.m. without knowing gen. configuration

cuticle



these } 2 layers (inner) are digitate + you need light microscope orientation to avoid "included" isolates i.e. section cutting digitate cell layer to give isolate appearance.

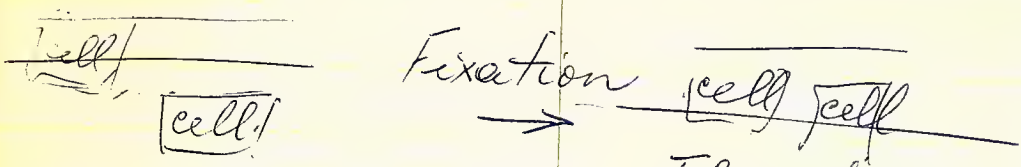
Bry. digestive tract etc. is U-shaped + also twists as you follow it along

Tentacles of bry. must be fixed when withdrawn within zooecium otherwise distortion due to pinocytosis.

Tentacles when withdrawn are not straight but a curved + in all birds

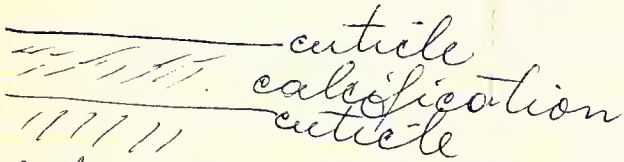
Bryozoan Anatomy — Fixation of Whole Specimens ①

Bry. wall cells have a lot of water & ∴ through fixation & embedding there is distortion of the cells — 2 layers of cells may be smeared to 1 layer.



If sectioned it appears as one layer of cell.

Bry walls may have several layers of cuticle — polylayer.



Polylayering will be diff. for each species ∴ know your partic. sp. cuticle & calcified material is commonly intermixed.

Need thin section of bry. examined under light microscope to det. orientation of cells & parts of bry —

Pages.

1-2,4. Bryozoan walls.
3 Keeping bryozoans alive in the lab.
3 Reference to general anatomy
4 Species found in dredged sample from Ile Louet
5 Differentiation- dedifferentiation in bryozoans.
6. Effects of oil pollution on bryozoans; Spine formation in bryozoans.
7-8 Embryological development in bryozoans.
9 Fixation. Ectoderm in bryozoans.
10 Dimorphism. Hybridization. Field trip to St. Pol.
11 - 14, 15. Differentiation of epithelial & peritoneum layers.
14,15,17. Funicular strand
16,17 Polypide formation
18-19 Staining & viewing specimens. Methylene blue (also discussion about funiculus). Cresyl blue stain. Bryozoan anatomy.
20. Sperular leucocytes; pigments in body wall; funiculus;brown bodies.
21-22 Vibracula; epithelial & peritoneum tissue; pore plates avicularium
23 Diaphragm, tentacular sheath. Menthol xls to relax bry. Mounting slides.
24 Incomplete description of process involved with methylene blue.
25 Caecum; spines.
26 & 29. Stains. Rouge neutre;cresyl blue; methylene blue.
27 Features of cyclostome tentacles.
28 Film on Growth of Membranipora membranacea.
30 Digestive tract.
31. E.M. sucrose-cacodylate "holding" solution.
33 Heterozooids (& cenozooids)
34 Avicularium on stained Bugula
35 Zooid anatomy
36 Pore plates
37 Muscle topography in zooid
38 Vestibular glands. Seasonality in bryozoans.
39 Budding in Membranipora.
40. Attachment of funiculus; position of anus
41 Vestibular glands; bacterial glands
42 Spermatids & eggs. Introcolony fertilization
43 Fixation & staining
44 Egg development (cleavage);larvae in ovicells; pseudopores.
45-52 Slides examined in Paris. Vestibular glands;brown body;anus; pore plates;bacterial glands; progression in vestibular glands. Development of spines.
47 Bacterial gland; regeneration; spermatozoa
48 Funiculus; egg development
49 Fixation; decalcification
50 Vestibular & bacterial glands
51 Pore plates; glands; muscles
52 Pore plates; E.M. photographs.

Front.

France (+ England)

1973. Bryozoan + field notes

IPAR.003198